Genomics of Zip Code Protein 1 (ZBP1)

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Abstract No. chan6842
Beamline(s): **X9B**

Localization of Beta-actin mRNA to the leading edge of fibroblasts requires the presence of conserved elements in the 3' untranslated region of the mRNA, including a 54-nucleotide element, which is termed the "zipcode". A protein was identified which binds to the proximal (to the coding region) half of the zip code with high specificity: ZBP1. ZBP1 is 576 amino acids weighing 63.271 kDa. Full-length ZBP1 was cloned into pMW172 (an IPTG inducible plasmid for protein expression in bacteria) utilizing EcoR1 and Nde1 restriction enzyme sites. Success of cloning was verified by Xmn1 restriction analysis as well as by DNA sequencing. Lysis was conducted in high salt - 1M NaCl, 0.1% Triton X-100 and 20 mM phosphate buffer plus protease inhibitors. Purification by cation and anion exchange followed by an S-200 sizing column produced multiple products as observed on SDS-PAGE gels. LC-MS analysis deconvoluted a main product at approximately 58 kDa. Gel shift assays on ZBP1 plus hot zip code RNA demonstrated ZBP1 to be an active zip code RNA binding protein.